Macrophage migration inhibitory factor as a redox-sensitive cytokine in cardiac myocytes

Masafumi Takahashi\textsuperscript{a,b,*}, Jun Nishihira\textsuperscript{c}, Masahisa Shimpo\textsuperscript{a}, Yuka Mizue\textsuperscript{e}, Shuichi Ueno\textsuperscript{a}, Hiroyuki Mano\textsuperscript{d}, Eiji Kobayashi\textsuperscript{b}, Uichi Ikeda\textsuperscript{a}, Kazuyuki Shimada\textsuperscript{a}

\textsuperscript{a}Division of Cardiology, Jichi Medical School, Tochigi, Japan
\textsuperscript{b}Division of Organ Replacement Research, Jichi Medical School, Tochigi, Japan
\textsuperscript{c}Central Research Institute, School of Medicine, Hokkaido University, Sapporo, Japan
\textsuperscript{d}Division of Functional Genomics, Jichi Medical School, Tochigi, Japan
\textsuperscript{e}Sapporo Immunodiagnostic Laboratory, Sapporo, Japan

Received 14 May 2001; accepted 5 July 2001

Abstract

Objective: Macrophage migration inhibitory factor (MIF), which plays a pivotal role in the control of inflammatory responses, was first characterized as a T-cell cytokine, but later was also found as a pituitary peptide released in response to infection and stress. However, MIF’s role and expression in the myocardium has never been reported. The goal of this study is to examine MIF in the myocardium.

Methods and results: MIF protein and mRNA levels were assayed using enzyme-linked immunosorbent assay (ELISA) and reverse transcription–polymerase chain reaction (RT–PCR), respectively. Increased MIF concentrations were detected in the sera of patients with acute myocardial infarction (AMI). In cultured rat cardiac myocytes, significant amounts of MIF were produced in response to hypoxia and hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), but not to angiotensin II, endothelin-1, interleukin-1β (IL-1β) or tumor necrosis factor α (TNFα). H\textsubscript{2}O\textsubscript{2}-induced MIF production increased in a time- and dose-dependent manner and was completely abolished in the presence of catalase. H\textsubscript{2}O\textsubscript{2} also induced MIF mRNA expression. The H\textsubscript{2}O\textsubscript{2}-induced MIF production was completely inhibited by the protein kinase C (PKC) inhibitor GF109203X, partially inhibited by the tyrosine kinase inhibitor herbimycin A, and uninhibited by calcium chelation or phorbol ester-sensitive PKC down-regulation. This suggests that H\textsubscript{2}O\textsubscript{2}-induced MIF production is mediated by an atypical PKC isoform. DNA microarray analysis revealed that 52 genes were preferentially expressed in response to MIF. Of these, the MIF-induced expression of both glutathione S-transferase (GST) and lipopolysaccharide-induced CXC chemokine (LIX) mRNAs was confirmed using RT–PCR analysis. Conclusion: The present results suggest that MIF is expressed by the myocardium in response to redox stress and may play a role in the pathogenesis of myocardial ischemia.

Keywords: Cytokines; Hypoxia/anoxia; Reperfusion; Ischemia

1. Introduction

Recent evidence indicates that inflammation plays an important role in the pathology of ischemic heart disease [1]. The inflammation in cardiovascular disease is associated with the activation of a variety of cells including lymphocytes, monocytes/macrophages, endothelial cells, smooth muscle cells and cardiac myocytes, which express and secrete proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor α (TNFα). These cytokines can modulate cardiac functions, cardiovascular remodeling, and development of thrombosis and atherosclerosis. The involvement of inflammation in ischemic heart disease was further demonstrated by the findings that elevated concentrations of C-reactive protein (CRP) [2], interleukin-6 (IL-6) [3], IL-8 [4], and TNFα [5] have a predictive value in the setting of acute coronary ischemia. Another important cardiac stress is reactive oxygen species (H\textsubscript{2}O\textsubscript{2}, OH\textsuperscript{-}, and O\textsubscript{2}\textsuperscript{-}) which generated from cardiac

myocytes in response to ischemia and during reperfusion after ischemia [6–8]. Recent evidence indicates that these reactive oxygen species can regulate intracellular signaling, and result in the modulation of cardiac functions similar to the cytokines [9].

Macrophage migration inhibitory factor (MIF) was originally identified as a T-cell-derived cytokine that inhibits macrophage migration and concentrates them at inflammatory loci [10]. We cloned rat MIF cDNA and also reported the physiological properties and structures of both human and rat MIF [11,12]. Although only activated T-cells were thought to produce MIF, recent reports have demonstrated MIF expression in a variety of cells, suggesting that it may play a role in other pathophysiological conditions [13]. MIF is also secreted as an anterior pituitary hormone and overrides the anti-inflammatory and immunosuppressive effects of glucocorticoids on macrophages and T-cells [14]. In addition, anti-MIF antibodies reduce inflammation in experimental models of glomerulonephritis, arthritis, allograft rejection, and septic shock, confirming the role of MIF in the regulation of inflammatory processes [15]. Elevated concentrations of MIF also were detected in the alveolar spaces of patients with adult respiratory distress syndrome (ARDS), the synovial fluid of patients with rheumatoid arthritis, and the serum of patients with atopic dermatitis [15]. With regard to cardiovascular disease, it has been more recently reported that MIF is expressed in atherosclerotic lesions [16,17]. However, the involvement of MIF in the heart has not been shown.

In the present study, we investigated whether MIF was expressed in patients with AMI and hypoxic myocardia. We demonstrated that the MIF serum concentration is elevated in patients with AMI, and that hypoxia and hydrogen peroxide (H₂O₂) stimulate MIF expression in cultured cardiac myocytes. In addition, DNA microarray analysis revealed MIF-responsive genes in cardiac myocytes. These findings suggest that MIF may function in the myocardium as a redox-sensitive cytokine.

2. Methods

2.1. Materials

Human IL-1β and TNFα were gifts from Otsuka Pharmacy, Tokushima, Japan. Human endothelin-1 (ET-1) was purchased from the Peptide Institute, Japan. PD098059 (final concentration: 10 μmol/l), SB203580 (10 μmol/l), herbimycin A (1 μmol/l), genistein (10 μmol/l), KT5270 (1 μmol/l), calphostin C (100 nmol/l), GF109203X (5 μmol/l), 2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA–AM) (30 μmol/l), and thapsigargin (1 μmol/l) were purchased from Calbiochem. The remaining reagents including EGTA (2 mmol/l) and phorbol 12,13-dibutyrate (PDBu) (1 μmol/l) were obtained from Sigma unless specified otherwise.

Polyclonal human and rat MIF antibodies were generated by immunizing New Zealand white rabbits with purified recombinant rat MIF as described previously [18,19]. The IgG fractions (4 mg/ml) were prepared using protein A Sepharose according to the manufacturer’s protocol.

2.2. Study subjects

MIF serum concentrations were measured in 10 healthy individuals (seven men and three women, mean age: 62.7±2.7 years) and in eight AMI patients (six men and two women, mean age: 62.8±2.9 years) hospitalized in Jichi Medical School Hospital within 6 h of the onset of symptoms. Patients who had pneumonia or other infectious diseases were excluded from this study. Blood samples were drawn from patients within 24 h of admission. Serum was separated immediately by centrifugation and stored at −80°C until use. All patients gave informed consent. The investigation conforms with the principles outlined in the Declaration of Helsinki.

2.3. Cell culture and hypoxia

Cardiac myocytes were prepared from ventricles of 1-day-old Sprague–Dawley rats as described previously [20]. Briefly, after dissociation with 0.25% trypsin, cell suspensions were washed with Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (FCS) and centrifuged at 500 g for 10 min. The centrifuged cells were then resuspended in 10% FCS containing DMEM. For selective enrichment of cardiac myocytes, the dissociated cells were preplated for 1 h, during which time noncardiac myocytes readily attached to the bottom of the culture dish. The resulting suspension of myocytes was plated onto 24-well dishes at a density of 1×10⁶ cell/ml. Thymidine (0.6 mg/ml) was added during the first 72 h to prevent proliferation of non-myocytes. Using this method, we routinely obtained enriched cultures containing >95% myocytes, as assayed by immunofluorescence staining with an anti-myosin heavy chain antibody. To expose the cells to hypoxia, they were placed in sealed modular incubation chambers and a constant stream of water-saturated 95% N₂/5% CO₂ was maintained over the culture as described previously [21].

2.4. MIF measurement

Human and rat MIF levels were determined with an enzyme-linked immunosorbent assay (ELISA) as described previously [22].
2.5. Reverse transcription–polymerase chain reaction (RT–PCR)

Total RNA was prepared from cardiac myocytes using the acid guanidine–phenol chloroform method. Reverse transcription was performed with Moloney murine leukemia virus reverse transcriptase using an oligo-dT primer, and subsequent amplification was with Taq DNA polymerase. The PCR was performed for 25 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min and extension at 72°C for 1 min using a thermal cycler (TP2000, Takara Biomedicals, Japan). During this analysis, the optimum number of cycles was used so as not to saturate the PCR products. The PCR primers used herein were as follows. Forward and reverse primers for rat MIF PCR were:

\[
5'\text{-CACCATG CCTATGTCATCGTGAACA-3'} \quad \text{and} \quad 5'\text{-GCCGGGCTCAAGGGAACCGTT-3'};
\]
for glyceraldehyde-3-phosphate dehydrogenase (GAPDH),

\[
5'\text{-TATGGCGCGCTGTCACCA-3'} \quad \text{and} \quad 5'\text{-CCACCTTCCTT G ATGTCATCA-3'};
\]
for glutathione S-transferase (GST),

\[
5'\text{-ATGTTGTTGATGATCCCGAC-3'} \quad \text{and} \quad 5'\text{-CCACCTTCCTT G ATGTCATCA-3'};
\]
for lipopolysaccharide (LPS)-induced CXC chemokine (LIX),

\[
5'\text{-GCTGATCTGACCAGTGCAAC-3'} \quad \text{and} \quad 5'\text{-GCTGATCTGACCAGTGCAAC-3'}.
\]

After PCR, an aliquot of each amplification mixture was subjected to electrophoresis on a 2% agarose gel, and DNA was stained with ethidium bromide. Bands were visualized and digitally photographed by use of a Luminescent Image Analyzer (LAS-1000, Fuji Photo Film Co.) and quantified by use of Image Gauge (version 3.0, Fuji Photo Film).

2.6. DNA microarray analysis

For gene screening in response to MIF in cardiac myocytes, we used DNA microarray analysis. RNA (2 μg each) was used to synthesize cDNA labeled with Cy3 or Cy5 (Amersham Pharmacia Biotech, Uppsala, Sweden), and hybridized to a cDNA microarray (IntelliGene Human Cancer CHIP; Takara Biomedicals, Shiga, Japan). To obtain expression profiles of Blast Bank samples, biotin-labeled complementary RNA (cRNA) was synthesized from the sample RNA (2 μg) by using the ExpressChip labeling system (Mergen, San Leandro, CA, USA), and allowed to hybridize with an oligonucleotide microarray (ExpressChip RO-1) that contained oligonucleotides based on a total of 1,152 genes. Hybridized slides were then incubated with streptavidin, anti-streptavidin first antibody, and finally Cy3-conjugated second antibody (all from Mergen) according to the manufacturer’s instruction. Detection of the signals and statistical analysis of the digitized data was carried out with a GMS 418 array scanner (Genetic Microsystems, Woburn, CA) and GeneSpring 3.2.2 software (Silicon Genetics, Redwood, CA), respectively. The gene list with GenBank accession numbers for our custom-made array can be obtained upon request.

2.7. Statistical analysis

Data are expressed as means±S.E.M. or S.D. For comparisons between multiple groups, we determined the significance of differences between group means by ANOVA using the least significant difference for multiple comparisons. Differences at values of \( P<0.05 \) were considered significant.

3. Results

3.1. Increased serum MIF concentrations in patients with AMI

To investigate whether MIF plays a major role in the human heart, we first measured plasma concentrations of MIF in healthy individuals (controls) and patients with AMI. As shown in Fig. 1, there was a statistically significant increase in the circulating concentrations of MIF in AMI patients compared with control subjects \((P<0.01)\). Median plasma concentrations of MIF were 3.0 ng/ml in controls and 12.5 ng/ml in patients with AMI. The data also correlated with the severity of AMI (maximum creatine kinase [CK] levels), but more cases are needed to confirm this trend.

3.2. MIF production in cardiac myocytes

Whether cultured rat cardiac myocytes can produce MIF was determined in culture medium using an ELISA for MIF. As shown in Fig. 2, hypoxia (3 and 6 h) and \( \text{H}_2\text{O}_2 \),
Fig. 2. Hypoxia and H$_2$O$_2$ stimulate MIF production in cardiac myocytes. Cardiac myocytes were stimulated by hypoxia (3 and 6 h), H$_2$O$_2$ (100 μmol/l), ET-1 (100 nmol/l), AII (100 nmol/l), IL-1β (10 ng/ml), and TNFα (10 ng/ml). After 6 h of incubation, the MIF concentration in the culture media was determined with an ELISA as described in Methods. Values represent means±S.D. of four independent experiments, each done in duplicate. *P<0.05 and **P<0.01.

(in contrast to vasoactive substances such as angiotensin II [AII] and ET-1, or inflammatory cytokines such as IL-1β and TNFα) were able to induce cardiac myocytes to produce significant levels of MIF.

H$_2$O$_2$-induced MIF production occurred within 1 h, reached a maximum after 6 h, and was substantially decreased by 24 h (Fig. 3A). Incubation with H$_2$O$_2$ for 6 h increased MIF production in a dose-dependent manner (10–100 μmol/l; Fig. 3B). In addition, this H$_2$O$_2$-induced MIF production was completely abolished in the presence of catalase (1000 U/ml). Similarly, incubation with H$_2$O$_2$ increased MIF mRNA expression in rat cardiac myocytes (Fig. 4).

3.3. Effects of various inhibitors on MIF production

We then examined whether protein kinases were involved in H$_2$O$_2$-induced MIF production in rat cardiac myocytes using tyrosine kinase inhibitors (genistein and herbimycin A), protein kinase C (PKC) inhibitors (GF109203X and calphostin C), mitogen-activated protein (MAP) kinase inhibitors (PD098059 for extracellular-regulated kinase [ERK] 1/2 and SB203580 for p38-MAP kinase), and a cyclic AMP-dependent kinase inhibitor (KT 5270). As shown in Fig. 5A, GF109203X and herbimycin A completely and partially inhibited H$_2$O$_2$-induced MIF production, respectively, whereas calphostin C, PD098059, SB203580, and KT 5270 had no significant effect. These results suggest that H$_2$O$_2$ induces MIF production through PKC, and partially through tyrosine kinase-dependent mechanisms in rat cardiac myocytes.

Because the PKC isoform is classified by sensitivity to calcium and phorbol ester, we examined the effects of calcium chelation and PKC down-regulation on H$_2$O$_2$-induced MIF production. As shown in Fig. 5B, neither chelation of intracellular calcium with BAPTA–AM plus EGTA nor depletion of internal calcium stores by thapsigargin inhibited MIF production induced by H$_2$O$_2$ [23]. To determine whether phorbol ester-mediated PKC activation is necessary for H$_2$O$_2$-induced MIF production, we
Fig. 4. H$_2$O$_2$ stimulates MIF mRNA expression in cardiac myocytes. Cardiac myocytes were stimulated by H$_2$O$_2$ (100 μmol/l) for the indicated periods. RT–PCR was performed as described in Methods. The RT–PCR products of GAPDH are shown as internal controls. (A) Electrophoresis showing MIF mRNA expression; (B) densitometric analysis of MIF mRNA expression. Results are representative of two independent experiments.

3.4. Gene expression in response to MIF

To investigate the role of MIF in myocardium, we used a DNA microarray to screen the MIF-responsive genes in rat cardiac myocytes. Of 1152 genes screened, 52 genes were preferentially expressed in response to MIF (Table 1). The MIF-induced increase of two of these genes, GST and LIX, was confirmed by RT–PCR analysis (Fig. 6).

4. Discussion

The major findings of this study were that (1) serum concentration of MIF was elevated in patients with AMI; (2) hypoxia and H$_2$O$_2$ stimulated MIF expression in
Table 1 cultured rat cardiac myocytes; (3) this MIF expression was completely inhibited by treatment with a specific PKC inhibitor GF109203X and partially inhibited by treatment with a tyrosine kinase inhibitor herbimycin A; and (4) MIF-induced expression of a number of cardiac myocyte genes. These findings suggest that MIF expression is due to redox stress via a PKC-dependent pathway in the myocardium and may play an important role in myocardial ischemia.

Inflammation has recently been shown to be associated with acute myocardial ischemia [1]. Several investigators reported elevated serum levels of CRP and inflammatory cytokines, such as IL-6, IL-8, and TNFα, in patients with unstable angina and AMI [3–5]. Because MIF also functions as a regulator of the inflammatory response, we examined serum levels of MIF during acute myocardial ischemia. AMI is associated with a rise in MIF plasma levels, the source of which is unknown. Prior studies on AMI revealed that several cytokines are preferentially produced by inflammatory cells in the peri-infarct zone and thus persistent cytokine elevations result from an increased infiltration of inflammatory cells [25]. More recently, Ridker et al. [5] reported that TNFα levels (elevated after AMI) could be a predictive risk factor for recurrent coronary events. In a recent experimental study of AMI induced by ligation of the left anterior descending coronary artery in rats, the expression of inflammatory cytokine genes including those for TNFα, IL-1β, and IL-6 was found to have increased in the myocardium [26]. Although T-cells, monocytes/macrophages, eosinophils, vascular endothelial cells, epithelial cells, adipocytes, anterior pituitary cells, and brain cells [13,15] are known cellular sources of MIF, the cardiac myocyte has not been a reported source. Our findings revealed that MIF is produced by rat cardiac myocytes in response to H₂O₂, suggesting that the myocardium is an important cellular source of MIF during acute myocardial ischemia.

The fact that hypoxia and H₂O₂ were able to stimulate MIF production in cardiac myocytes is of particular interest. We and other investigators have previously reported that TNFα stimulates MIF production in various cell types [13,14], but not in cardiac myocytes. Furthermore, IL-1β, AII, and ET-1 were also unable to stimulate MIF production, suggesting that MIF is strictly a redox-sensitive cytokine, and that another signaling pathway is responsible for MIF expression in cardiac myocytes.

It is well known that there is a significant release of reactive oxygen species in ischemia and during reperfusion after ischemia [6–8]. However, the pathways of redox-sensitive intracellular signaling have not been defined. We showed that MIF production induced by H₂O₂ is completely inhibited by GF109203X, suggesting that redox-sensitive MIF production is mediated through a PKC-dependent mechanism. Interestingly, another well-known PKC inhibitor, calphostin C, had no effect on H₂O₂-induced MIF production. This difference may be based on...

<table>
<thead>
<tr>
<th>Name</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P-450 isozyme 5 (P-450 IVB2)</td>
<td>15.3</td>
</tr>
<tr>
<td>Glutathione S-transferase</td>
<td>9.1</td>
</tr>
<tr>
<td>Insulin-like growth factor-binding protein</td>
<td>8.4</td>
</tr>
<tr>
<td>Serine protease inhibitor</td>
<td>7.9</td>
</tr>
<tr>
<td>Cyclic nucleotide-gated channel</td>
<td>6.9</td>
</tr>
<tr>
<td>CXC chemokine LIX</td>
<td>6.4</td>
</tr>
<tr>
<td>Branched chain α-keto acid dehydrogenase El-subunit (BCKDHB)</td>
<td>6.2</td>
</tr>
<tr>
<td>KRAB/zinc finger suppressor protein (KS1)</td>
<td>6.1</td>
</tr>
<tr>
<td>α-1B adrenergic receptor</td>
<td>6.0</td>
</tr>
<tr>
<td>Interleukin-4 receptor</td>
<td>5.8</td>
</tr>
<tr>
<td>Nucleotide diphosphate kinase type</td>
<td>5.3</td>
</tr>
<tr>
<td>Atrial natriuretic peptide clearance receptor</td>
<td>5.2</td>
</tr>
<tr>
<td>Amiloride sensitive Na⁺ channel protein</td>
<td>5.1</td>
</tr>
<tr>
<td>10-formyltetrahydrofolate dehydrogenase</td>
<td>5.0</td>
</tr>
<tr>
<td>Solute carrier family family</td>
<td>4.7</td>
</tr>
<tr>
<td>Calcium channel, voltage-dependent, γ-subunit</td>
<td>4.6</td>
</tr>
<tr>
<td>Protein kinase C-binding protein NELL</td>
<td>4.5</td>
</tr>
<tr>
<td>Voltage-gated Ca²⁺ channel</td>
<td>4.6</td>
</tr>
<tr>
<td>Solute carrier family 16 (monocarboxylic acid transporters)</td>
<td>4.5</td>
</tr>
<tr>
<td>Kruppel-like transcription factor</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Listed in this table are the genes preferentially expressed (top 20 genes).

Fig. 6. MIF stimulates the expression of GST and LIX mRNA in cardiac myocytes. After cardiac myocytes were stimulated by MIF (3 μmol/l) for 24 h, RNA was extracted, and RT–PCR was performed as described in Methods. The RT–PCR products of GAPDH are shown as internal controls. Results are representative of two independent experiments.
the mechanisms of GF109203X and calphostin C action. GF109203X inhibits PKC by competitive inhibition of the ATP-binding site [27]. On the other hand, calphostin C inhibits PKC by interaction with the protein’s regulatory domain, which is the binding site of diacylglycerol and phorbol esters [28]. Because H₂O₂-induced MIF production is mediated through phorbol ester-insensitive PKC, these results are reasonable.

In fact, it has been shown that hypoxia and H₂O₂ directly stimulate activation of several PKC isoforms in the myocardium [29] and that the PKC inhibitor GF109203X has cardioprotective effects [30], suggesting that PKC may play an important role in hypoxia/reperfusion injury. Because MIF regulates cell cycles and survival in other cell types [15], we speculate that MIF is associated with PKC-mediated myocardial injury. The PKC isoform is generally classified into conventional PKC (α, β, γ), novel PKC (δ, ε, η, θ), and atypical PKC (ζ, λ) based on calcium- and phorbol ester-sensitivities. The fact that MIF production by H₂O₂ was not affected by calcium chelation or phorbol ester treatment suggests that an atypical PKC isoform is responsible for MIF production in the myocardium. In this regard, Takeishi et al. [29] demonstrated that H₂O₂ activates an atypical PKC isoform independent of phospholipase C and tyrosine kinase signaling. Nevertheless, hypoxia-mediated activation of PKC isoform is mediated through pathways involving phospholipase C and tyrosine kinase. Tyrosine kinase(s) is also involved in redox-sensitive signal transduction in the myocardium. H₂O₂-induced MIF production is partially, but significantly, inhibited by herbimycin A, suggesting the involvement of tyrosine kinase(s) in this MIF production. Takeishi et al. [31] more recently reported that myocardial ischemia stimulates activation of c-Src tyrosine kinase and a novel MAP kinase, big MAP kinase1 (BMK1), in perfused pig hearts. Because BMK1 is a redox-sensitive kinase and c-Src is an upstream regulator of BMK1 activation [32], it is speculated that the c-Src-BMK1 pathway may also be involved in H₂O₂-induced MIF production in the myocardium. Taken together, atypical PKC and c-Src tyrosine kinase are candidate signaling molecules for H₂O₂-induced MIF expression in the myocardium. Further investigations are required to elucidate the signaling pathways of redox-sensitive MIF production in the myocardium.

Because MIF’s role in the myocardium is unknown, we used a DNA microarray to screen for and found the differential expression of genes responding to MIF in cardiac myocytes. Interestingly, the expression of GST and LIX is clearly MIF-induced. GST plays a key role not only in detoxification, but also as an antioxidant. Locki et al. [33] demonstrated that MIF is similar to two GST classes (μ and θ), suggesting that MIF plays an important role in the redox stress system. The gene for LIX, a novel neutrophil-chemoattractant CXC chemokine, was cloned as a glucocorticoid-attenuated response gene [34]. Although LIX message is abundantly expressed in various tissues, LPS-induced LIX expression is strongest in the heart [35]. In contrast to LIX, other CXC chemokines, such as KC or macrophage inflammatory protein-1 (MIP-2), which are also expressed abundantly, have a different pattern of expression, suggesting that MIF may stimulate the accumulation and recruitment of neutrophils via LIX in myocardial ischemia.

In conclusion, we showed that the serum concentration of MIF is elevated in patients with AMI and that MIF expression is stimulated by hypoxia and H₂O₂ in cardiac myocytes. Furthermore, DNA microarray analysis revealed a number of MIF-responsive genes in cardiac myocytes, suggesting that MIF may function in the myocardium as a redox-sensitive cytokine.

Acknowledgements

We thank Ms Toshiko Kambe for her technical assistance. This study was supported by the Ministry of Education, Culture, and Science of Japan (No. 40296108), Japan Research Foundation for Clinical Pharmacology and the Jichi Medical School Young Investigator Award.

References

[11] Nishihiro J, Kuriyama T, Sakai M et al. The structure and physico-